

PATENT  
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Docket 093/005p

### CLAIM AMENDMENTS

1. ~~(Currently Amended) A cell population comprising at least 60% differentiated cells that are progeny of primate pluripotent stem (pPS) cells, and~~  
A differentiated cell population as part of a system for obtaining hepatocyte lineage cells, wherein the system comprises:

a) primate pluripotent stem (pPS) cells isolated from a primate blastocyst and cultured in vitro; and

b) the population of differentiated cells, obtained by differentiating said isolated pPS cells; and wherein at least about 60% of the cells in the differentiated cell population have at least three of the following characteristics:

- antibody-detectable expression of  $\alpha_1$ -antitrypsin (AAT);
- antibody-detectable expression of albumin;
- absence of antibody-detectable expression of  $\alpha$ -fetoprotein;
- RT-PCR detectable expression of asialoglycoprotein receptor (ASGR);
- evidence of glycogen storage;
- evidence of cytochrome p450 activity;
- evidence of glucose-6-phosphatase activity; and
- the morphological features of hepatocytes +

~~wherein the cell population is cultured or maintained in a medium containing a histone deacetylase inhibitor so as to maintain said characteristics.~~

2. ~~(Currently Amended) A cell population comprising at least 60% differentiated cells that are progeny of primate pluripotent stem (pPS) cells, and~~  
A population of hepatocytes obtained by differentiating isolated human embryonic stem (hES) cells,

wherein the cell population is characterized as being a population of hepatocytes on the basis that at least about 60% of cells have at least three of the following characteristics:

- antibody-detectable expression of  $\alpha_1$ -antitrypsin (AAT);
- antibody-detectable expression of albumin;
- absence of antibody-detectable expression of  $\alpha$ -fetoprotein;
- RT-PCR detectable expression of asialoglycoprotein receptor (ASGR);
- evidence of glycogen storage;
- evidence of cytochrome p450 activity;
- evidence of glucose-6-phosphatase activity; and
- the morphological features of hepatocytes;

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wherein the ~~differentiated cells~~ hepatocytes are karyotypically normal and non-malignant,  
and wherein the cell population comprises less than 0.1% endothelial cells or Kupffer cells.

3. *(Original)* The cell population of claim 1, wherein at least about 60% of the cells have at least five of said characteristics.
4. *(Original)* The cell population of claim 1, wherein at least about 80% of the cells have at least seven of said characteristics.
5. *(Original)* The cell population of claim 1, wherein the level of cytochrome p450 enzyme 1A1/1A2 activity is at least as high as in primary human adult hepatocytes.
6. *(Original)* The cell population of claim 1, wherein the pPS cells are embryonic stem cells.
7. *(Original)* The cell population of claim 6, wherein the embryonic stem cells are human.
8. *(Original)* The cell population of claim 7, which is essentially free of undifferentiated hES cells.
9. *(Currently amended)* A method for obtaining the cell population of claim 1, comprising culturing ~~cells from the stem cell line~~ the pPS cells in a growth environment that comprises a hepatocyte differentiation agent which is a histone deacetylase inhibitor.
10. *(Original)* The method of claim 9, wherein the hepatocyte differentiation agent is n-butyrate.
11. *(Original)* The method of claim 9, comprising pre-differentiating the cells by forming embryoid bodies, or by pre-differentiating the cells by culturing in a medium containing dimethyl sulfoxide (DMSO), dimethylacetamide (DMA); hexmethylene bisacetamide, or another polymethylene bisacetamide.
12. *(Original)* The method of claim 1, comprising further culturing the cells in a medium containing a cytokine or hormone selected from glucocorticoids, epidermal growth factor (EGF), insulin, TGF- $\alpha$ , TGF- $\beta$ , fibroblast growth factor (FGF), hepatocyte growth factor (HGF), IL-1, IL-6, IGF-I, IGF-II, and HBGF-1.

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13. *(Currently amended)* A method for obtaining the cell population of claim 1, comprising culturing ~~cells from the stem cell line~~ the pPS cells in a growth environment that comprises one or more hepatocyte maturation factors that are either:
- a) an organic solvent selected from dimethyl sulfoxide (DMSO), dimethylacetamide (DMA); hexmethylene bisacetamide, and other polymethylene bisacetamides; or
  - b) a cytokine or hormone selected from glucocorticoids, epidermal growth factor (EGF), insulin, TGF- $\alpha$ , TGF- $\beta$ , fibroblast growth factor (FGF), hepatocyte growth factor (HGF), IL-1, IL-6, IGF-I, IGF-II, and HBGF-1.
14. *(Currently amended)* A method of screening a compound for hepatocellular toxicity, comprising combining ~~a cell~~ a differentiated cell population according to claim 1 with the compound, and determining whether the compound is toxic to the cell.
15. *(Currently amended)* A method of screening a compound for its ability to modulate hepatocellular function, comprising combining ~~a cell~~ a differentiated cell population according to claim 1 with the compound, determining any phenotypic or metabolic changes in the cell that result from contact with the compound, and correlating the change with an ability to modulate hepatocellular function.
16. *(Currently amended)* The method of claim 15, comprising determining whether the compound changes enzyme activity or enzyme secretion ~~by the cell~~ in the cell population.
17. *(New)* The cell population of claim 1, cultured or maintained in a medium containing a histone deacetylase inhibitor.
18. *(New)* The cell population of claim 17, wherein the histone deacetylase inhibitor is n-butyrate.
19. *(New)* The cell population of claim 2, wherein at least about 80% of the cells have at least seven of said characteristics.
20. *(New)* A method of screening a compound, comprising combining the hepatocytes of claim 2 with the compound, and determining whether the compound is toxic to the cells, or whether there are phenotypic or metabolic changes in the cells that result from contact with the compound.